Effect on the Wheatgrass Somaclone Inducement by Genotype and Culture Medium

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The XXI International Grassland Congress / VIII International Rangeland Congress took place in Hohhot, China from June 29 through July 5, 2008.

Proceedings edited by Organizing Committee of 2008 IGC/IRC Conference

Published by Guangdong People's Publishing House

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Key words: Wheatgrass (*Agropyron* Gaertn.), somaclone, embryonic age, genotype, medium

Introduction Successful application of plant biotechnology for plant improvement requires the development of efficient plant regeneration systems from cultured cells or tissues. The objective of this study was to determine the optimal conditions for producing morphogenic callus and plant regeneration from immature embryos of wheatgrass and to establish an efficient tissue culture system for wheatgrass for use in fundamental studies, genetic transformation and *in vitro* plant propagation (Liu Gongshe et al., 2004).

Materials and methods Three varieties of wheatgrass (Agropyron Gaertn.) were used: *A. mongolicum* cv. Keng (Keng) (2n=4x=28), *A. desertorum* cv. Nordan (Nordan) (2n=4x=28) and hybrid wheatgrass *A. cristatum* × *A. desertorum* cv. Hycrest Mengnong (Mengnong) (2n=4x=28). Immature embryos (15 days after anthesis) were used as explant in tissue culture for plant regeneration. Three callus initiation media were used including: (1) MSC medium containing MS media+CH (500 mg/L)+2,4-D (2.0 mg/L); (2) N6C medium containing N6 medium+CH (500 mg/L)+2,4-D (2.0 mg/L); and (3) MNC medium containing MN medium+CH (500 mg/L)+2,4-D (2.0 mg/L). MN culture mediums increased Ca^{2+}, K^{+} on the basis of MS and N6 culture medium.

Results The effect of inoculating immature embryos of the three wheatgrass genotypes (Keng, Nordan and Mengnong) on three callus induction media (MSC, N6C and MNC) is shown in Table 1. The quantity and quality of the callus induced were different. All callus rates exceeded 80% with the highest rate of 99% occurring when immature embryos of Nordan were inoculated on MSC medium (but the callus quality was poor as the majority were soft and white). When immature embryos of Keng were inoculated on MSC medium, the percentage of embryogenic callus reached 47% and the callus were compact, yellow (Figure 1) Analysis of variance of the embryogenic callus rate data showed that genotype, media and genotype×media were all significant, being in the order of genotyoe>genotype×media>culture media (Table 2).

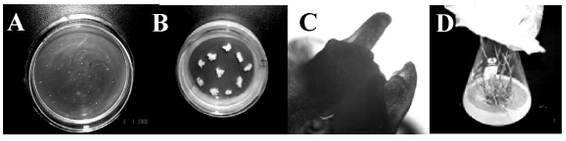

Figure 1 In vitro *culture of Keng on MSC*
A. Immature embryos inoculated on MSC; B. Embryogenic calli; C. Embryoid; D. Plant regeneration.

Table1 *The influence of genotype and medium on immature embryo culture of wheatgrass.*

Genotypes	M	NI	NC	FC	NE	FE
Keng	MNC	100	93	93	20	22
	MSC	100	86	86	40	47
	N6C	100	92	92	37	40
Mengnong	MNC	100	92	92	0	0
	MSC	100	90	90	15	17
	N6C	100	90	90	0	0
Nordan	MNC	100	84	84	31	37
	MSC	100	99	99	26	26
	N6C	100	96	96	14	15

Note: Media=M; No. of immature embryos=NI;
No. of callus=NC; Frequency of callusing=FC;
No. of embryogenic callus=NE;
Frequency of embryogenic callus=FE

Table 2 *ANOVA of co-treatment with genotype and medium on the embryogenic callus rate.*

SV	SS	DF	MS	F-value	Pr>F
G	7221.73	2	3610.87	126.16**	0.0001
M	1139.73	2	569.87	19.91**	0.0001
G×M	2593.33	4	648.33	22.65**	0.0001
E	1030.40	36	28.62		
S	11985.20	44			

Note: ** $p<0.01$
Source of variation=SV; Genotype=G; Medium=M;
Genotype×Medium=G×M; Error=E; Sum=S

Conclusions A strong relationship was shown by the research to exist between genotype and culture medium. The best combination for immature embryo culture of wheatgrass is Keng×MSC medium because the bright yellow compact callus was the perfect embryoid.

References

Liu Gongshe, Qi Dongmei, 2004. Study of immature embryos of several species of Leymus Via in vitro culture. *Acta Prataculturae Sinica* 13(1), 70-73.